View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

RESEARCH NOTE

Evaluation of eight commercial tests for *Mycoplasma pneumoniae* antibodies in the absence of acute infection

R. Nir-Paz, A. Michael-Gayego, M. Ron and C. Block

Department of Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

ABSTRACT

Eight commercially available tests for *Mycoplasma pneumoniae* (Serodia-Myco II, Labsystems IgM and IgG EIA, IgM and IgG LISA tests, Immuno-Well IgG test and SeroMP IgM and IgG) were compared using 204 single sera from healthy individuals. IgM peaked in late childhood and then declined, while IgG rose progressively into adulthood. Inter-assay agreement was poor. Positivity in Serodia-Myco II and LISA IgG was associated with blood group or Coombs positivity, suggesting non-specific reactions. The study confirmed that single serum serology is unsuitable for the diagnosis of *M. pneumoniae* infection, and that commercially available tests need further improvement.

Keywords Assays, diagnosis, IgG, IgM, *Mycoplasma pneumoniae*, serology

Original Submission: 2 June 2005; **Revised Submission:** 8 October 2005; **Accepted:** 10 December 2005

Clin Microbiol Infect 2006; 12: 685–688
10.1111/j.1469-0691.2006.01469.x

Mycoplasma pneumoniae is one of the most common causes of pneumonia [1], but culture of *M. pneumoniae* is performed rarely because of the lengthy growth period and low yield [2,3]. Other diagnostic modalities, such as PCR, are available, but many protocols are in use and standardisation is lacking [4]; commercial molecular diagnostic kits have not yet been properly evaluated. For many decades, confirmation of *M. pneumoniae* infection has been based on serological tests. The reference method, i.e., the complement fixation assay, is time-consuming and technically demanding [5]. Moreover, some reports have suggested that *M. pneumoniae* infection is not recognised in a proportion of patients, even if paired acute and convalescent sera are examined [3,5,6]. A range of commercially available serological kits has replaced the complement fixation test in most laboratories [2,7]; however, for most patients, only one serum sample is available. Indeed, most kits include a definition for acute infection using a single serum sample. Previous studies have evaluated commercially available kits using either positive paired sera or sera from patients with a positive nucleic acid amplification result by PCR or nucleic acid sequence-based amplification [6,8–10]. One study from France [8] suggested that positive IgM and IgG results varied according to the kit used and the age of the patient. Another compared serological assays in blood donors, but did not compare the tests in relation to age or other potentially significant variables [11]. Data concerning the performance of these assays in healthy populations are scarce. Therefore, the present study compared the frequency and age associations of positive single test results obtained using several commercially available serological kits for healthy individuals.

Sera were drawn from samples scheduled for disposal at the Hadassah Hospital blood bank (Jerusalem, Israel) during the period January–June 2004. The sera had been obtained either from blood bank donors (aged ≥18 years) or from patients admitted for elective surgical procedures (patients aged 0–18 years). The study was approved by the institutional ethics committee. Data available included age, gender, blood type and auto-antibodies against erythrocytes. At least 20 samples were collected in the age groups 0–0.5, 0.6–2, 3–6, 7–12, 13–18, 19–26, 27–40, 41–65 and >65 years. A few sera were discarded because of bacterial contamination, small volume or turbidity, leaving a total of 204 sera (17–33 samples/age group). Duplicate sera from individual patients were avoided. Only patients with no indication of febrile disease during the 2 weeks preceding admission were admitted for elective surgery. For blood donors, the required disease-free period is at least 30 days.

Corresponding author and reprint requests: R. Nir-Paz, Department of Molecular and Cell Biology, 510 Barker Hall, University of California, Berkeley, CA 94720-3202, USA
E-mail: nirpaz@berkeley.edu

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **12**, 672–694

Eight different serological tests were evaluated: Serodia-Myco II (Fujirebio, Tokyo, Japan), Labsystems *M. pneumoniae* IgM and IgG EIA (Ani Labsystems, Helsinki, Finland), *M. pneumoniae* IgM and IgG LISA tests (BMD SA, Marne La Vallee, France), ImmunoWell *M. pneumoniae* test (GenBio, RUST, Germany; this kit uses the same materials as the LISA kit, but applies lower cut-offs for adults), and SeroMP IgM and IgG tests (Savyon Diagnostics, Ashdod, Israel). All tests were performed and interpreted according to the manufacturers' instructions, and were reported as negative, equivocal or positive. Statistical analysis was with SPSS software v.12.0.0 (SPSS Inc., Chicago, IL, USA). Cohen's kappa test was used for comparing agreement between tests. The chi-square test or Fisher's exact test, as appropriate, were used to analyse differences in proportions, with $p < 0.05$ considered significant.

The numbers of positive, equivocal and negative results varied among the different tests as follows: Serodia-Myco II, 32, 26 and 146; Labsystems *M. pneumoniae* IgM, 54, 60 and 90; Labsystems *M. pneumoniae* IgG, 173, 14 and 17; *M. pneumoniae* LISA IgM, 40, 9 and 155; *M. pneumoniae* LISA IgG, 34, 54 and 116; ImmunoWell IgG, 115, 47 and 42; SeroMP IgM, 16, 21 and 167; and SeroMP IgG, 108, 19 and 77. Age had a major influence on the proportion of positive sera (Fig. 1). All kits showed peaks of positive IgM tests for the 6–26-year age group in at least 20% of sera. IgG levels increased with age, and remained high even in the elderly. The decline in the number of positive results with the LISA IgG assay for adults reflects the higher cut-off point for this kit.

Inter-test agreement was very poor (Table 1). The highest kappa value was *c.* 0.6 for the SeroMP assay and the ImmunoWell IgG. Most other values were well below that, even for the same immunoglobulin. Positive or equivocal results were generally not associated with blood type, Rhesus factor type, Coombs reaction or gender origin of the sera tested, except that the Serodia kit tended to give equivocal or positive results with Coombs-positive sera (8/12, p 0.005). Unequivocal positivity also correlated with a positive Coombs reaction (5/12, p 0.011) for this kit. The same tendency was observed with the LISA IgG with Coombs-positive sera (9/12, p 0.033 for non-negative test results; 5/12, p 0.017 for clearly positive results). With the LISA IgG assay there was also a significant association between blood group B and positive

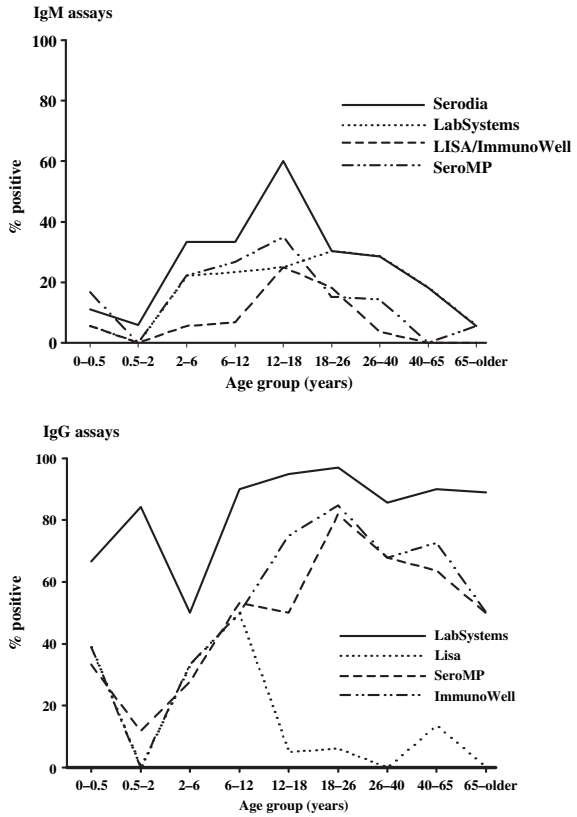

Fig. 1. Distribution of positive results according to the different assays tested: IgM and IgM-like assays (Serodia) and IgG assays.

or equivocal sera (63.4% of group B sera vs. 38.0% of other groups; $p < 0.003$). This association was also significant for unequivocally positive sera (26.8% of B group sera vs. 16.1% of other groups; $p < 0.029$). While this suggested that blood group B might produce some false-positive reactions with this kit, another explanation might be the cut-off calculation applied in this test, since the same association was not observed with the ImmunoWell IgG assay, which uses the same plates and reagents. Interestingly, the LISA IgM and Labsystems IgM gave more positive results with females than with males (27.3% vs. 14.2%, p 0.021, and 34.8% vs. 20.4%, p 0.021, respectively).

The study showed that age was associated with test positivity in healthy individuals, with the IgM peaking at primary/secondary school age, and declining thereafter. The high IgM positivity in these age groups casts doubt on the suggestion that combining IgM tests with amplification-based tests in the paediatric population might be of benefit [12–14]. Moreover, elevated IgM does

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **12**, 672–694

Table 1. Agreement of performance among different tests according to Cohen's kappa test

(a) According to positive, equivocal and negative results

	Labsystems IgM	Labsystems IgG	LISA IgM	LISA IgG	ImmunoWell IgG	SeroMP IgM	SeroMP IgG
Serodia	0.303[a]	0.030[b]	0.287[a]	0.173[a]	0.119[a]	0.381[a]	0.120[c]
Labsystems IgM		0.079[c]	0.288[a]	0.187[a]	0.200[a]	0.190[a]	0.168[a]
Labsystems IgG			0.023[b]	0.030[b]	0.228[a]	0.024[b]	0.217[a]
LISA IgM				0.091[d]	0.113[a]	0.377[a]	0.094[d]
LISA IgG					0.280[a]	0.140[c]	0.184[a]
ImmunoWell IgG						0.063[d]	0.455[a]
SeroMP IgM							0.105[c]

(b) According to negative and non-negative (positive + equivocal) results

	Labsystems IgM	Labsystems IgG	LISA IgM	LISA IgG	ImmunoWell IgG	SeroMP IgM	SeroMP IgG
Serodia	0.278[a]	0.055[d]	0.380[a]	0.229[a]	0.171[a]	0.499[a]	0.229[a]
Labsystems IgM		0.141[a]	0.361[a]	0.247[a]	0.242[a]	0.278[a]	0.263[a]
Labsystems IgG			0.029[b]	0.076[d]	0.288[a]	0.027[b]	0.211[a]
LISA IgM				0.122[b]	0.123[a]	0.451[a]	0.080[b]
LISA IgG					0.229[a]	0.198[a]	0.308[a]
ImmunoWell IgG						0.109[a]	0.553[a]
SeroMP IgM							0.137[c]

(c) According to positive-only results

	Labsystems IgM	Labsystems IgG	LISA IgM	LISA IgG	ImmunoWell IgG	SeroMP IgM	SeroMP IgG
Serodia	0.450[a]	0.038[b]	0.293[a]	0.277[a]	0.144[c]	0.349[a]	0.095[b]
Labsystems IgM		0.047[b]	0.450[a]	0.229[a]	0.269[a]	0.285[a]	0.141[d]
Labsystems IgG			0.029[b]	0.042[b]	0.311[a]	0.030[b]	0.315[a]
LISA IgM				0.109[b]	0.175[a]	0.396[a]	0.090[b]
LISA IgG					0.268[a]	0.060[b]	0.189[a]
ImmunoWell IgG						0.088[c]	0.595[a]
SeroMP IgM							0.066[b]

[a] $p \leq 0.001$.
[b] $p > 0.05$, not significant.
[c] $0.001 < p \leq 0.01$.
[d] $0.01 < p \leq 0.05$.

not necessarily reflect recent exposure, since the period from any disease to serum sampling was at least 30 days for most samples. IgG positivity persists into adulthood, with a small decline in the elderly. Preliminary results from a new test [15] suggest lower rates of positive results among blood bank donors (2–4% IgM and 40–50% IgG), but age distributions were not reported. Previous suggestions [8,16] that positive results are more prevalent in adults led [8] to the age-related change of cut-off values in the LISA *M. pneumoniae* IgG assay, although this change was not implemented in the ImmunoWell IgG assay, which uses the same materials. The present data suggest that such cut-off adjustments might not suffice. Poor agreement among different assays has also been noted previously [6–9,11], and the low kappa values suggest that some tests will give misleading results.

As in other countries [16,17], the main exposure to *M. pneumoniae* in the Israeli population occurs during school age, resulting in at least 80% of adults having antibodies (Fig. 1). Nevertheless, there is an intriguing 6-year interval during which the IgG peak lags behind the increase in IgM levels. This might reflect the need for exposure to more than one subtype to produce a sufficient immune response, or for multiple exposures to the same pathogen. Overall, this study emphasises the need to use paired sera for the diagnosis of *M. pneumoniae* infections, as well as the need for more accurate and reliable diagnostic kits.

ACKNOWLEDGEMENTS

The authors thank A. Mousa and S. Mudahi-Orenstein of the Hadassah-Hebrew University Medical Center Blood Bank for their help in collecting the sera used in this study, and A. Ron

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **12**, 672–694

for graphical assistance. This work was supported in part by a research grant from Savyon Diagnostics Ltd, and by a Joint fellowship of the Hebrew University Medical School and the Hadassah Hebrew University Medical Center, both awarded to R. Nir-Paz.

REFERENCES

1. Marston BJ, Plouffe JF, File TM *et al*. Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance study in Ohio. The Community-Based Pneumonia Incidence Study Group. *Arch Intern Med* 1997; **157**: 1709–1718.
2. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin Microbiol Rev* 2004; **17**: 697–728.
3. Daxboeck F, Krause R, Wenisch C. Laboratory diagnosis of *Mycoplasma pneumoniae* infection. *Clin Microbiol Infect* 2003; **9**: 263–273.
4. Loens K, Ieven M, Ursi D *et al*. Detection of *Mycoplasma pneumoniae* by real-time nucleic acid sequence-based amplification. *J Clin Microbiol* 2003; **41**: 4448–4450.
5. Waites KB, Bebear CM, Robertson JA, Talkington DF, Kenny GE. *Laboratory diagnosis of mycoplasmal infections*. Washington, DC: American Society for Microbiology, 2001.
6. Talkington DF, Shott S, Fallon MT, Schwartz SB, Thacker WL. Analysis of eight commercial enzyme immunoassay tests for detection of antibodies to *Mycoplasma pneumoniae* in human serum. *Clin Diagn Lab Immunol* 2004; **11**: 862–867.
7. Thacker WL, Talkington DF. Analysis of complement fixation and commercial enzyme immunoassays for detection of antibodies to *Mycoplasma pneumoniae* in human serum. *Clin Diagn Lab Immunol* 2000; **7**: 778–780.
8. Petitjean J, Vabret A, Gouarin S, Freymuth F. Evaluation of four commercial immunoglobulin G (IgG)- and IgM-specific enzyme immunoassays for diagnosis of *Mycoplasma pneumoniae* infections. *J Clin Microbiol* 2002; **40**: 165–171.
9. Templeton KE, Scheltinga SA, Graffelman AW *et al*. Comparison and evaluation of real-time PCR, real-time nucleic acid sequence-based amplification, conventional PCR, and serology for diagnosis of *Mycoplasma pneumoniae*. *J Clin Microbiol* 2003; **41**: 4366–4371.
10. Beersma MF, Dirven K, van Dam AP, Templeton KE, Claas EC, Goossens H. Evaluation of 12 commercial tests and the complement fixation test for *Mycoplasma pneumoniae*-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the 'gold standard'. *J Clin Microbiol* 2005; **43**: 2277–2285.
11. Csango PA, Pedersen JE, Hess RD. Comparison of four *Mycoplasma pneumoniae* IgM-, IgG- and IgA-specific enzyme immunoassays in blood donors and patients. *Clin Microbiol Infect* 2004; **10**: 1094–1098.
12. Waris ME, Toikka P, Saarinen T *et al*. Diagnosis of *Mycoplasma pneumoniae* pneumonia in children. *J Clin Microbiol* 1998; **36**: 3155–3159.
13. van Kuppeveld FJ, Johansson KE, Galama JM *et al*. 16S rRNA based polymerase chain reaction compared with culture and serological methods for diagnosis of *Mycoplasma pneumoniae* infection. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 401–405.
14. Dorigo-Zetsma JW, Zaat SA, Wertheim-van Dillen PM *et al*. Comparison of PCR, culture, and serological tests for diagnosis of *Mycoplasma pneumoniae* respiratory tract infection in children. *J Clin Microbiol* 1999; **37**: 14–17.
15. Konig G, Bottcher M, Christiansen G *et al*. *Mycoplasma pneumoniae*-ELISA medac: new assays for the detection of specific IgG, IgA, and IgM antibodies (abstract 115). *Proceedings of the 15th Congress of the International Organisation for Mycoplasmology*. Athens, GA: International Organisation for Mycoplasmology, 2004: 83.
16. Aubert G, Pozzetto B, Hafid J, Gaudin OG. Immunoblotting patterns with *Mycoplasma pneumoniae* of serum specimens from infected and non-infected subjects. *J Med Microbiol* 1992; **36**: 341–346.
17. Foy HM, Kenny GE, Cooney MK, Allan ID. Long-term epidemiology of infections with *Mycoplasma pneumoniae*. *J Infect Dis* 1979; **139**: 681–687.

RESEARCH NOTE

Genetic variation of coxsackie virus B5 strains associated with aseptic meningitis in Greece

A. Papa, K. Dumaidi, F. Franzidou and A. Antoniadis

A' Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Greece

ABSTRACT

In order to explore the genetic relationships among coxsackie virus B5 strains in Greece, the nucleotide sequences of the partial VP1 gene in strains isolated from aseptic cases of meningitis were determined and compared with those of strains isolated from other countries. Phylogenetic analysis showed a high degree of divergence (25%) among Greek strains isolated in different years, which clustered with high bootstrap values in a different subgroup of viruses, suggesting that enterovirus types vary with time rather than geographical distribution. A non-synonymous mutation present in the strains of this study was not observed in other coxsackie virus B5 strains.

Corresponding author and reprint requests: A. Papa, A' Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece
E-mail: annap@med.auth.gr

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **12**, 672–694